THE PARASITIC ACTINOMYCETES AND OTHER FILAMENTOUS MICROÖRGANISMS OF THE MOUTH

A Review of Their Characteristics and Relationships, of the Bacteriology of Actinomycosis, and of Salivary Calculus in Man

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CONTENTS

This review, one of a series on the bacteriology of the human mouth (113, 114), deals with a group of gram-positive, filamentous microörganisms, both branched and unbranched, which are cultivable under partial anaerobiosis or in the presence of carbon dioxide. The group comprises the branched genus *Actinomyces* and the unbranched genus *Leptotrichia*, which are dealt with in detail; while certain other microörganisms, to which the generic names *Actinomyces* and *Leptotrichia* (or "*Leptothrix*") have been applied, are discussed in an effort to clarify their hitherto confused relationships. The etiology and pathogenesis of actinomycosis in man, and the nature and manner of formation of salivary calculus or tartar, are considered with special reference to these microörganisms.

THE PARASITIC ACTINOMYCETES[1]

The actinomycetes are gram-positive microörganisms characterized by the formation of a mycelium or network of branched and rebranched filaments. Mycelium formation is a property of the true fungi, and the actinomycetes are often classed with the fungi rather than with the bacteria proper. The

[1] For the purposes of this discussion, the terms "parasitic actinomycete" and "actinomyces" are employed interchangeably as common names for organisms of the genus *Actinomyces*. The common name "actinomycete" is employed here in its customary inclusive sense. The terms "parasitic" and "saprophytic" are employed in the following sense: "parasitic" = living on or in another organism and deriving nourishment therefrom; "saprophytic" = living on dead organic matter. It may be noted that neither of these terms implies pathogenicity. Many parasitic microörganisms produce disease, but some, e.g., the white staphylococci of the skin or the pigmented *Neisseria spp.* of the throat, are not known to do so. Conversely, most saprophytes lack disease-producing capacity, but the clostridia of gas gangrene are a notable exception.

group, however, shows a wide range of variation within itself, and the members of it with which this review is concerned are clearly much more bacteria-like than fungus-like. On the whole, the actinomycetes may best be considered as intermediate between the bacteria proper and the true fungi, or, in the words of Waksman (143), as "an independent group of organisms which is closely related to the bacteria through some of the constituent forms, but which has adopted a fungus-like form of growth."

This review deals with only one subdivision of the actinomycetes, those of strictly parasitic habit, which are included here under a single specific name, *Actinomyces israeli*. The natural habitat of these organisms appears to be the mouth and throat. They are not found in nature apart from the tissues of man and animals, or otherwise under saprophytic conditions, and their properties are such as to indicate their incapacity to multiply or even to survive under such conditions. They appear, in other words, to be obligate parasites. They seem ordinarily to be harmless, but under exceptional circumstances they give rise to actinomycosis, a disease of which they are the principal cause both in man and in animals. These parasitic actinomycetes should be distinguished sharply from the broad and varied category of *saprophytic actinomycetes*, with which this paper is concerned only for incidental and comparative purposes (see table 1). The saprophytic organisms inhabit the soil, and are widely distributed on grains and grasses. This group includes both pathogenic and non-pathogenic members. It may be emphasized that the relationship between the saprophytic and the parasitic actinomycetes depends on two points of resemblance only. Both are composed of a gram-positive, branched mycelium, and both may be pathogenic for man and animals and produce lesions in which radially clubbed "sulfur granules" are found. The saprophytic forms, on the other hand, are recovered only rarely from true actinomycosis ("lumpy jaw") in cattle, and their association with true actinomycosis in man seems to be even more unusual. They are found, however, in certain tropical skin diseases ("mycetomas") of both man and animals.

The generic name *Actinomyces* ("ray fungus") was originally given by Harz (56) to organisms observed in material from lumpy jaw in cattle. It was Harz likewise who applied the name actinomycosis to this disease. These terms have come to be used nearly universally for the disease and for its most common causative agent, the parasitic actinomycete. Unfortunately the term *Actinomyces* has also been used for quite distinct members of the saprophytic group. This confusing double usage will be resolved if the recent recommendation of Waksman and Henrici (145) achieves general acceptance. After several attempts had been made to distinguish the saprophytic and parasitic groups by reserving the generic name *Actinomyces* for the former and using for the latter such names as *Cohnistreptothrix* (107, 143) or *Actinobacterium* (110), Waksman and Henrici (145) have now applied the generic term *Actinomyces* exclusively to the parasitic organisms with which this review deals, while the genus of aerobic non-sporebearing forms, formerly called *Proactinomyces*, is now *Nocardia*, and the genus of aerobic forms with spores in chains on aerial hyphae, previously designated *Actinomyces*, is now *Streptomyces*.

The full name *Actinomyces israeli*, applied to the parasitic forms, accords well with international usage. The specific name is credited to Kruse by the Argentine workers, Negroni and Bonfiglioli (102), who have adopted it, as

TABLE 1

Comparison of the Parasitic and Saprophytic Actinomycetes

	PARASITIC ACTINOMYCETES	SAPROPHYTIC ACTINOMYCETES*
Natural habitat	Mouth and throat of man and probably of cattle and other animals; obligate parasites; sometimes pathogenic.	Soil, grains and grasses; widely distributed in nature; some pathogenic species, but most forms are non-pathogenic.
Cellular morphology	Branched mycelium, gram-positive, not acid-fast. Marked tendency to fragment into bacillary forms.	Branched mycelium, gram-positive; some are acid-fast. Generally little tendency to fragment into bacillary forms.
Character of growth	Bacteria-like colonies without aerial hyphae; no spores; no pigments.	Colonies more mold-like, often with aerial hyphae and spores (conidia); many produce yellow, orange or black pigments.
Temperature requirements	Optimum, 37 C; no growth at 22 C.	Optimum usually 15–20 C.
Relation to oxygen	Oxygen tolerance limited; generally fail to grow or grow poorly under aerobic conditions.	Aerobic; some forms do not grow anaerobically.
Metabolism	Probably never proteolytic. Ferment carbohydrates with production of acid.	Many forms actively proteolytic; may utilize carbohydrates without acid production.
Species recognized	One only: *Actinomyces israeli*. (Provisional; heterogeneous but not yet satisfactorily subdivided.)	Many, subdivided into several families.
Pathogenicity	Causative agent of true actinomycosis in man and animals.	Occasional causes of an actinomycosis-like disease, very rare in man, and of tropical cutaneous mycetomas, e.g., Madura disease.

* Further details of the saprophytic actinomycetes are given by Henrici (58), St. John-Brooks (120), Erikson (42), and Waksman (143).

has Grootten (50) in France, Puntoni (110) in Italy, and Waksman (143) in this country. It seems best to discard the names *Actinomyces bovis* and *A. hominis*, since both have been used so loosely as to require the modifying phrase, "Wolff-Israel type" to make them apply without ambiguity to the parasitic

form. The name *Actinomyces israeli* is appropriate in view of the fact that
Israel (61) was the first to recognize its parasitic nature, and since Wolff and
Israel (152) first cultivated it from actinomycosis and laid the foundation for
our present knowledge of the etiology and pathogenesis of that disease.

Actinomyces israeli

Parasitic actinomycetes with the characteristics described below have been
isolated from actinomycosis in man, cattle and other animals by Wolff and Israel
(152), Wright (153), Colebrook (21, 22), Lignières (81) and many others.
Wright predicted in 1905 that they would be found as a normal inhabitant of
the mouth. Lord (84, 85) demonstrated them in sections of carious detritus
and in material from tonsils; and they have since been isolated from the human
mouth and throat by many workers (97, 99, 86, 41, 130, 126), who have shown
these organisms to be very similar to or indistinguishable from those of ac-
tinomycosis.

Morphology. *A. israeli* is a gram-positive, branching, filamentous organism.
It is not acid-fast, does not form spores, and is non-motile. The individual fila-
ments are generally less than 1 micron in width, like most bacteria, and unlike
the true fungi, which are usually several times as wide. *A. israeli*, however,
varies markedly in appearance under different conditions. Since some of the
difficulties in this field seem to have been determined in the past by failure to
recognize or to identify this organism, a full description of it seems warranted
here.

In tissue sections made from the lesions of actinomycosis, the organism appears
in the form of compact granules or colonies which are often visible to the un-
aided eye. The characteristics of the granule are frequently clear enough for
diagnostic purposes under low magnification (50 to 100 diameters), but their
details are best seen under higher power (400 diameters or more). The granule
may be roughly circular or irregular in outline, or may be seen to consist of
several colonies of different size and shape which have coalesced. Each granule
is composed of a dense reticulum of fibrils which may stain irregularly in hema-
toxylin-eosin preparations but take the violet dye in sections stained by Gram's
method. Around the periphery of the granule the ends of individual filaments
may be seen projecting, or, more characteristically, part or all of the periphery
may be composed of the radially arranged hyaline *clubs* to which the actinomy-
cetes owe their name. The clubs take the eosin stain; they are several times
wider than the filaments whose ends they enclose, and the filament can some-
times be traced within the structure of the club. Such radial clubs are often
regarded as primary diagnostic features of the true actinomycotic granule,
but their value for the purpose is only relative, since granules composed of
A. israeli may fail to show clubs, and, as noted below, similar if not identical
clubs may be found in other disease processes, notably actinobacillosis.

In exudates from actinomycosis, colony particles are frequently but not always
present, and may be macroscopically visible as *sulfur granules*,—irregularly
spherical or mulberry-like masses, usually whitish or pale yellow, from a fraction

of a millimeter to about 2 mm in diameter. The granules are usually soft and easily broken under light pressure, but they may occasionally be tough or even calcified. In a wet, unstained slide-coverslip preparation the crushed granule appears as a more or less disorganized mass of irregular, bent and branching filaments, some of which may terminate in the characteristic clubs. In preparations fixed and stained by Gram's method the structure of the granule is usually completely lost; clubs are not in evidence, and although some individual branching filaments may appear, the typical picture is that of a mass of irregular, bent gram-positive rods with projecting portions which give the fragments a *twig-like* appearance.

In preparations from the mouth or throat in the absence of actinomycosis, these organisms may take several forms. Clubs are not seen, and clearly branched filaments are seldom apparent. Lord (85) has described the occurrence of branching filaments in tissue sections of scrapings from carious teeth and of masses taken from tonsillar crypts; and Naeslund (99), Soderlund (128) and others have described a similar picture, with occasional clubs, in sections of salivary calculus. Clumps of bent twig-like rods, usually with little or no clear evidence of dendritic branching, can often be seen in stained films made from these locations, or from gingival scrapings from a pyorrheal pocket. That such organisms are in fact *A. israeli* is indicated by their recovery in cultures from such sources; but microscopic examination is seldom sufficient in itself to justify their identification. Darkfield examination of wet preparations is an excellent method for this purpose, since the clumps retain their character more perfectly in wet preparations than when dried for staining, and the darkfield method shows the continuity of structure of the twig-like and branched forms. On the other hand, evidence from culture preparations suggests that *A. israeli* may also occur on the oral and pharyngeal mucous membranes as unbranched gram-positive rods or diphtheroid forms which would defy identification by means of the microscope alone.

In cultures, the morphology of *A. israeli* runs the gamut from a compact mass clearly composed of a branching mycelium of gram-positive filaments to a quite undistinguished picture of regular short rods which may be evenly stained or granular, and which show no indication of branching. These differences are associated with roughness or smoothness of colony form. Rough colonies, whether they grow on an agar surface, in the depths of an agar shake culture or in broth, show branching forms regularly when prepared for microscopic examination with care to avoid disorganization of the mass. Broken twig-like forms, however, are much more common than long filaments. Intermediate and smooth colonies often yield a picture that resembles that of the diphtheria bacillus, with granular and polar-stained forms disposed in V- and Y-groupings, and with suggestive but not conclusive evidence of branching. Some smooth colonies, derived after repeated subculture from rough and clearly branched forms, may appear as short evenly stained rods with no distinguishing characteristics. The rough and intermediate forms often show terminal swellings or "clubbed forms" like those of the diphtheria bacillus; but the true clubs

seen in sections of actinomycotic tissue do not appear in either wet or fixed microscopic preparations made from cultures of *A. israeli*. Wright (153) obtained clubs irregularly in cultures by growth or persistence in the presence of high concentrations of serum or ascitic fluid. Lord and Trevett (86) were unable to produce clubs by similar means. It has been generally assumed that club formation represents a response of the tissues to the presence of the organism. Bayne-Jones (4), on the other hand, obtained typical eosin-staining clubs in sections of a colony from a glucose broth culture.

Growth and Metabolism. The isolation and maintenance of pure cultures of *A. israeli* have often been found difficult, apparently only because two characteristics of the organism have not been widely recognized:

1. Strains are apt to die out if cultivated successively on any single medium, but generally thrive if transferred alternately to different media (43, 115).

Among the media recommended, in addition to infusion broth or agar containing 1 per cent of glucose, have been Dorset's egg or glycerin-egg medium (43), Lubinski's medium (102), chopped-meat infusion broth, and Bacto brain-heart infusion to which 2 per cent of agar is added (115). None of these media appears to be fully satisfactory, in itself; hence the need for alternate transfers, apparently on the "varied diet" principle.

2. The organism has a *limited tolerance for oxygen* which varies from strain to strain and in individual strains at different times. Some strains grow only under anaerobic conditions, while others may grow aerobically, especially in broth; but any strain is likely to be lost if maintained through successive transfers under aerobic conditions only. Rosebury, Epps and Clark (115) were able to maintain 15 strains without difficulty by cultivation in anaerobic jars containing 5 per cent of carbon dioxide, which was found to favor continued growth.

A. israeli grows best at 37°C and fails to grow at 22°C. The optimum pH range for growth is 7.2 to 7.6 (21, 101). According to Negroni and Bonfiglioli (102) no growth occurs in the absence of carbohydrate; but other workers have obtained growth in plain nutrient broth or agar, and the writer has cultivated the organism successfully in fluid media negative to Benedict's reagent. Growth is much more abundant, however, in the presence of glucose.

Acid without gas is produced from a wide range of carbohydrates (81, 60, 102, 43). Naeslund (97) stated that some strains showed definite proteolytic action on coagulated egg albumin and serum and on gelatin, in the presence of sterilized saliva and glucose; but other workers have found *A. israeli* uniformly non-proteolytic (81, 86, 102, 43). Lignières (81) reported no production of indole, while Negroni and Bonfiglioli (102) found indole produced in small amounts, and also recorded reduction of nitrate with two strains but not with others. According to these workers, H_2S is formed in glucose agar containing liver extract and lead acetate; and washed human red blood cells are hemolyzed, but not rabbit cells or whole blood. Most workers have found *A. israeli* non-hemolytic. Pigments or antimicrobial substances such as have been obtained from species of saprophytic actinomycetes (see Waksman, 144) have not been recovered from this organism.

A. israeli is easily killed by moderate heating: in 3 to 10 minutes at 62 to 64°C (153); in 1 hour at 60°C (21); or in 30 minutes at 60 to 65°C (102). Wright (153) reported that granules in actinomycotic pus were still viable after being dried in test tubes in air for 18 to 22 days. Cultures dried over anhydrous phosphoric acid in vacuum by Negroni and Bonfiglioli (102) remained alive for 3 or 4 months in the ice chest. Lyophilization or similar methods of preservation do not seem to have been tried with this organism. Freshly isolated cultures in agar shake tubes, kept in the refrigerator, should be transferred every two weeks, while older strains may remain viable in this medium in the cold for about a month.

Cultural Characteristics. The appearance of vigorously growing *A. israeli*, as noted above, varies in relation to the roughness or smoothness of the strain. When first isolated from parasitic or pathological sources in man most if not all strains are either rough or intermediate. Some strains show little tendency to change, but others become smooth after prolonged artificial cultivation. The rough strains are the more distinctive.

In glucose-agar shake cultures incubated in air at 37°C, growth is seldom visible within 48 hours and more commonly requires from 3 to 6 days. The colonies are white or greyish, roughly spherical or compound, "crumb-like" and up to about 1.5 mm in diameter. The appearance of rough strains in this medium is generally highly characteristic and hence of diagnostic value; growth is concentrated in a zone about 5 mm wide, with its upper limit from 0.5 to 2 cm below the free surface. Scattered colonies may be present below and occasionally above this zone, but growth does not occur on the exposed surface. With smooth strains there may be no zone of concentrated growth, but uniformly distributed colonies may extend from the bottom of the tube to a level 0.5 to 1 cm from the surface, at which the growth terminates abruptly. When a whole colony of a rough strain in this medium is transferred with a capillary pipette to a slide, the colony is often found to be tough and difficult to break up and emulsify; but either darkfield or gram-stained preparations show the characteristic compact branched mycelium.

In glucose broth, incubation in air at 37°C may be successful through one or more subcultures; but anaerobic incubation is more reliable. In either instance the growth of rough strains is again very characteristic: they appear as white or greyish masses, up to about 5 mm in diameter, at the bottom of the tube, the medium itself remaining perfectly clear. Colebrook (21) has aptly described these masses as resembling tiny cauliflowers or breadcrumbs. Like other colonies of rough strains they are often difficult to break up. Transfer of the supernatant medium to a fresh culture medium fails to secure growth; it is necessary to transfer a visible fragment of the colony. Intermediate strains tend to grow as smaller particles or granules either at the bottom or along the side of the tube, or as viscid or flocculent masses, again with little or no general turbidity; while smooth strains, particularly under anaerobic conditions, may produce uniform turbidity with or without a viscid or granular sediment.

Surface colonies on suitable media, such as glucose agar or brain-heart agar, ncubated anaerobically with 5 per cent CO_2 for 4 to 6 days, are sufficiently

distinctive with rough or intermediate strains to make this the method of choice for the isolation of *A. israeli*, particularly from contaminated sources. On sparsely seeded plates the colonies may have a diameter as great as 3 mm, but more commonly they are 1 mm in diameter or less. To the naked eye they appear dead white, or more rarely slightly greyish or yellowish. They are opaque, matt, and somewhat pitted or irregular in both surface and outline. Under a magnification of 5 to 10 diameters, and with suitably reflected illumination, rough colonies present a glistening but irregular surface and a high-raised contour, "heaped-up" in Erikson's (43) phrase, somewhat like colonies of the tubercle bacillus, but on a smaller scale. Such colonies are usually found to be strongly adherent to the medium, so that they are hard to remove with an inoculating needle, often come away all in one piece, and are emulsified with difficulty. Intermediate colonies may have a smooth but irregularly contoured surface which, with their white color and peculiar opacity, may give them a striking resemblance to the crown of a human molar tooth, as noted by Sullivan and Goldsworthy (130). Completely smooth colonies, on the other hand, are not distinctive, but resemble the smooth round raised colonies of white staphylococci or diphtheroids. They are soft and easily broken and emulsified. Whether such smooth actinomyces colonies ever appear on primary plates from contaminated sources is not known; if they do it would not be easy to mark them apart from other colonies on the plate, and it would be particularly difficult to distinguish them from certain diphtheroids. It may be noted that some of the common aerobic bacteria, particularly streptococci, may appear on anaerobic-CO_2 plates in colonies very different from the familiar ones on aerobic media; and an occasional rough white colony similar to those described above may turn out on examination to be a streptococcus.

Subdivision of A. israeli. Several attempts have been made to subdivide the parasitic actinomycetes into distinct groups, but none of the proposed classifications has been generally accepted, perhaps because the number of strains studied by any one investigator has never been large enough to make reported differences seem convincing. The bases for subdivision have been differences in (a) oxygen tolerance (97); (b) morphology and growth rate (41); (c) fermentation reactions and agglutination (60); and (d) colony morphology, correlated with source of strains and with agglutination reactions (43, 78). Naeslund (97) and Emmons (41) recognized that their groups were closely interrelated. Holm's (60) two groups seemed to be distinct, but he studied only 9 strains, all derived from actinomycosis in man. Lentze (78) and Erikson (43) found independently that fermentation reactions were too variable to be useful for classifying strains derived from actinomycosis in both man and cattle. These workers reported that bovine strains were usually smoother than human strains and that they were distinct by agglutination. Erikson, moreover, found 5 bovine strains to be more tolerant of oxygen than her 15 strains from human disease, and also less exacting in their need for CO_2. Strains of *A. israeli* isolated from mucous membranes of persons without actinomycosis were not included in these studies. Such strains seem to be smooth more often

than those obtained from actinomycosis in man (115). Smoothness or rough-
ness is, however, a dubious basis in itself for classification. It appears on the
whole that *A. israeli* is a heterogeneous species; but until more extensive data
are available it seems unwise to attempt to subdivide it. Data given below
show that strains from all three common sources, actinomycosis in man and
in cattle, and non-actinomycotic mucous membranes, may all be capable of
producing actinomycosis under experimental conditions.

Interrelationships of A. israeli with Other Microörganisms

Taxonomic Position of the Actinomycetes. The whole order *Actinomycetales*
exemplifies, perhaps as well as any biological group, the continuity of the living
world, and the fluidity of the boundaries which are drawn in an effort to sepa-
rate it into larger or smaller categories. Sometimes called "higher bacteria,"
they seem to be related through the diphtheria bacillus, the tubercle bacillus,
and the closer relatives of both, to the bacteria proper; and through the true
fungi to the plant kingdom as a whole. Because of their apparent intermediate
position the writer has refrained from speaking of them either as bacteria or
as fungi.

Actinomyces and Lactobacilli. The parasitic actinomycetes may have an
additional bond of relationship to the bacteria proper, through lactobacilli of
the bifidus type (114). The lactobacillus isolated from the intestines of guinea
pigs by Crecelius and Rettger (27) seems particularly reminiscent of an ac-
tinomycete intermediate between the rough and smooth forms described above,
in its morphology, its nonfastidious anaerobic habit, and in other respects.
The only direct comparison between *A. israeli* and *Lactobacillus bifidus* that
seems to have been made is that of Puntoni (109), who reported that differ-
ences between the two groups in morphology and in biochemical and serological
characteristics were quantitative rather than qualitative and lay within the
range of expected variation of the two heterogeneous groups. This subject
seems worth further study.

Parasitic and Saprophytic Actinomycetes. The parasitic actinomycetes are
ordinarily so clearly distinct from the saprophytic forms that there would seem
to be little reason to confuse them. Two points of difficulty have nevertheless
arisen, as follows:

1. Members of the *saprophytic* group have been observed in and isolated
from actinomycosis, especially in cattle (14, 81) and they have also been culti-
vated from the human mouth (97, 125). Naeslund (100), moreover, has found
that rare strains of saprophytic actinomycetes—two strains out of several
hundred isolated from plants and soil—were capable of producing actinomycosis-
like disease in experimental animals. None of 30 saprophytic strains isolated
from mucous membranes of human subjects without actinomycosis had this
property. Two other instances are cited by Colebrook (22) and a third by
Wright (154) in which saprophytic actinomycetes seem to have been responsible
for suppurative lesions containing typical granules. In an earlier study Naes-
lund (97) had been able to recover *A. israeli* from the human mouth in greater

abundance than the saprophytic forms; and in view of the greater resistance and widespread distribution in nature of these latter, he argued that they are chance invaders and not indigenous to the mouth. He noted that 3 out of 30 saprophytic strains isolated from the mouth grew poorly at 37°C but luxuriantly at 15 to 20°C,—a clear indication that these strains, at all events, were not indigenous.

According to the data collected by Colebrook (22) the truly aerobic (saprophytic) actinomycetes have been recovered in only a small minority of instances from actinomycosis in cattle, and rarely if ever from the disease in man (1 doubtful case out of 94). Cope (25) notes that the occasional recovery of saprophytic actinomycetes from lesions in man may be due to contamination, but believes that they may at times cause such disease. Biggart (12) has recorded a case of generalized and rapidly fatal disease following hysterectomy, from which a true saprophytic actinomycete was recovered. The organism produced small non-fatal lesions in experimental animals. No granules were found either in the patient at autopsy or in the animals inoculated with cultures.

The facts thus do not justify categorical exclusion of the saprophytic actinomycetes as incitants of actinomycosis; but it seems clear that their importance in the human disease is of a very low order.

2. The use of the term *anaerobic actinomyces* as an overall designation for the parasitic forms (e.g., Colebrook, 23) seems to have led to confusion in that some strains whose characteristics place them with *A. israeli* have not been classified as such because they were found able in some degree to grow in air. Anaerobiosis within the parasitic group is relative and variable, and its variation does not afford a basis for their subdivision. The group as a whole is easily distinguished from the aerobic saprophytic group by other characteristics (table 1). Organisms which seem to have been *A. israeli* but were able to grow in air, and which were regarded as ambiguous either by the original author or by others, have been described by Naeslund (97), Lord and Trevett (86), Bibby and Knighton (11), Crowley (28), and Bartels (3).

The relationship to actinomyces of organisms described under this and other names, including *Leptothrix* and *Cladothrix*, is discussed below.

THE LEPTOTRICHIA

The genus *Leptotrichia* comprises certain *unbranched* gram-positive rods and filaments which occur characteristically and prominently as parasites of the human mouth, but which have no known pathogenic or other significance. Only a single species, *Leptotrichia buccalis*, can be defined with any assurance. The names *Leptotrichia* and *Leptothrix* have however been used widely and very loosely to apply to a variety of forms, some of which can be placed with little doubt in distinct genera, while others have remained unidentified.

The name *Leptothrix* is not properly applied to this group. This term was first given by Kützing in 1843 (76) to a genus of filamentous iron bacteria which are free living and autotrophic, and cannot be considered even distantly related to the parasitic filaments under discussion. These parasitic forms were

first described as *Leptothrix buccalis* by Robin (111, 112). Similar microörganisms had been described and pictured by Leeuwenhoek in his letter of 1683 (36) and by Buehlmann in 1840 (17). Trevisan, in 1879 (139), recognized the distinction between Kützing's iron forms and those of the mouth, and proposed the generic name *Leptotrichia* for the latter.

Leptotrichia buccalis is a gram-positive unbranched filamentous organism, non-motile, non-sporulating, with a tendency to grow as coarse, often granular threads or long rods which may be rounded and sometimes clubbed at one end and tapered or pointed at the other. The most complete study of this organism is that of Thjøtta, Hartmann and Bøe (137). Clear descriptions of it have also been given by Kligler (72), who called it *Leptothrix buccalis*, and by Wherry and Oliver (149), who gave it the name *Leptothrix innominata* as used earlier by Miller (95).

According to Thjøtta and his collaborators this organism can always be seen in smears from the mouth, especially from the central part of the dorsum of the tongue. They succeeded in growing it on Bacto brain-heart infusion medium containing up to 2 per cent of agar, and found that it grew better "in a jar emptied of air and filled up with CO_2" than under either aerobic or completely anaerobic conditions. The previous workers had described it as a facultative anaerobe or microaerophile. Colonies are about 1 mm in diameter after 24 hours, but continue to grow and reach a much greater size after 7 days' incubation at 37°C. They are described as having a characteristic thatched or medusa-head appearance and a pearly translucency and lustre, and are found by microscopic examination to consist of "long streamers of rods or filaments closely packed together like rafts of timber in a river." On blood agar the colonies are smaller and more irregular; they are not hemolytic but may produce slight greening. Although distinctly gram-positive in young cultures they become increasingly gram-negative later, and often show gram-positive granules in a gram-negative base. They stain yellow with iodine. All strains have been found to ferment several carbohydrates including starch, and to acidify milk with or without coagulation. Final pH values ranged from 4.7 to 5.2. Gelatin was not liquefied, and neither indole nor H_2S was produced; but some strains reduced nitrates. Complement fixation tests with 5 rabbit antiserums showed ten strains to be mutually interrelated but not homogeneous; while a strain of oral *Lactobacillus acidophilus* did not react with any of the serums (137).

Kligler (72) believed this organism to be one of three that constitute the essential flora of early dental caries, the others being *Cladothrix placoides* (see below) and *L. acidophilus*. So far as this view relates to *Leptothrichia buccalis*, it was based chiefly on the abundance of the organism in deposits from early carious lesions and on its filamentous character, which suggested a capacity to help fix the more essential decalcifying organisms to the tooth surface. *L. buccalis* was found to be less active in acid production and decalcification than either of the other forms, and did not augment their powers in these respects when grown in combination with either or both. Kligler's view with regard

to this organism has not received confirmation, and there seems to be little reason at present to credit it.

Leptotrichia have also been associated with salivary calculus, as noted below; but descriptions under this name, or under the name *Leptothrix*, of organisms isolated from tartar have never conformed clearly with that given above. In some instances, notably the *Leptothrix buccalis* of Bulleid (18, 19), the forms isolated from tartar can be identified with little doubt as *Actinomyces israeli*. Otherwise the identity of organisms isolated from tartar and given the names *Leptotrichia* or *Leptothrix* remains uncertain. This statement applies even to the work of so careful a student of the actinomycetes as Naeslund (99). This author's description of "leptothrix" isolated from tartar fails to identify it clearly, although it seems probable that the organism was *not* an actinomycete, particularly since typical *A. israeli* was also described as having been recovered from the same source. The filamentous organisms isolated from tartar and other oral sources by Bibby and his co-workers (8, 9, 11) are also inadequately identifiable. Some of these seem to have been *A. israeli*, but to have represented a selection of more oxygen-tolerant strains; while others, described as branching leptotrichia, cannot be placed with any assurance in either group. Organisms isolated both from tartar and from excised gingival tissue by Grythe (52, 53, 54) seem likewise to have included *A. israeli* and possibly saprophytic actinomycetes as well; but other forms described as "leptothrix" are of doubtful identity. There seems consequently to be no evidence that true *Leptotrichia buccalis* occurs in salivary calculus, and no reason, despite the frequent use of this and similar names in relation to these concretions, to assign any rôle to this organism in its deposition.

Other attempts have been made to impute pathogenic significance to the oral leptotrichia, but without convincing evidence (see MacKenzie, 88A).

MISCELLANEOUS FILAMENTOUS MICROÖRGANISMS

Several apparently unrelated groups of microörganisms have been either (a) described under other names although they may belong with the parasitic actinomyces, or (b) described as actinomycetes or leptotrichia although they seem to belong in neither group.

Possible Actinomycetes Described Under Other Names. As noted above, organisms described as *Leptotrichia buccalis*, or loosely as leptothrix or leptotrichia, have sometimes been identifiable as *Actinomyces israeli*.

Leptotrichia placoides (Bergey et al., 5) may include either *A. israeli* or a rough lactobacillus, or possibly a form intermediate between the two; while some of the properties attributed to it seem to exclude it from both these groups. Dobrzyniecki (37, 38) first applied the name *Leptothrix placoides alba* to a gram-positive aerobic organism isolated from a root canal. It grew in chains of rods and tangled threads, failed to form spores, liquefied gelatin and blood serum, and grew at 16 to 18°C as well as at 37°C. Kligler (72) cultivated 58 strains which he believed to correspond with Dobrzyniecki's organism, and named it *Cladothrix placoides*. Kligler's strains were not uniform. Some were

gram-negative, and some liquefied gelatin. Most of them, however, were gram-positive and non-proteolytic. They fermented glucose, sucrose and in some instances lactose; reduced nitrates, and failed to form ammonia or indole. Morphologically they were variable, with coccoid, diphtheroid and filamentous forms, some of which showed swollen ends and "false" branching. The preponderant gram-positive non-liquefying form may have been an actinomycete which was not observed under conditions that permitted demonstration of true branching, although Kligler's statement that this form was aerobic and grew slowly at 20°C makes it doubtful that the organism was *A. israeli*. Bibby and Knighton (11) considered Kligler's organism to be the same as forms described by them, which in turn seem to have been selected aerobic strains of *A. israeli*; these organisms were non-proteolytic, but also grew slowly at room temperature.

Kligler regarded his *Cladothrix placoides* as second in importance to *Lactobacillus acidophilus* in the flora of early dental caries. This view was based in part on the common occurrence of the organism in the disease, along with lactobacilli and *Leptotrichia buccalis*; and also on the finding that *Cladothrix placoides* was second to *Lactobacillus acidophilus* in acidogenic power, all other species tested having been much less active. It is this finding which suggests that *C. placoides* may have been a rough lactobacillus; and there is nothing in Kligler's description of the majority of his strains that would definitely contraindicate this view.

Other So-called Leptothrices and Actinomyces. The names *Leptothrix* and *Actinomyces* have been applied rather indiscriminately to several additional microorganisms, not all of which can be identified from published descriptions, but none of which appear to fit into either of these genera.

As early as 1890, Miller (95) remarked that the name *Leptothrix buccalis* had been applied loosely, and this has continued to be true. Miller's own use of this generic term is confusing. His *Leptothrix buccalis maxima* and *Leptothrix innominata* may both have been true *Leptotrichia buccalis*. His *Bacillus buccalis maximus* has been identified by Goadby (47) and by Kligler (72) as an aerobic spore-bearing form belonging in the *Bacillus subtilis* group. Miller's *Leptothrix gigantea*, described, like the others, only on the basis of microscopic study, may have been a true fungus, as Thjøtta, Hartmann and Bøe (137) have suggested.

Leptothrix racemosa is a name given by Vicentini (142) to a peculiar structure, seen in smears from the mouth, consisting of a long thick rod or filament with "conidia" or spore-like bodies clustered around one end. These forms may also have been fungi, or, as Thjøtta and his co-workers believed, artefacts produced by agglomeration of cocci around a leptotrichial or other filament. A somewhat similar form described by Beust (6, 7), likewise on the basis of smears only, consisted of fusiform elements attached to a central stalk so as to give a "test-tube brush" appearance. Beust called this form *Leptothrix falciformis*. Such structures have also been seen by Davis (30) in so-called tonsillar granules and by Goodrich and Moseley (48) and others in pyorrheal pus; and the writer

has seen them occasionally in dark-field preparations of gingival scrapings. An organism with similar morphology was cultivated by Mendel (94) and named *Leptothrix asteroide*. This was a strictly anaerobic gram-negative organism which was said to be pathogenic for animals. Mendel's description and photomicrographs suggest a member of the genus *Bacteroides*. Tunnicliff (140) and Tunnicliff and Jackson (141) isolated from tonsillar granules what seems to have been the same organism, which they named *Vibriothrix tonsillaris*,—a motile gram-negative anaerobe which in pure culture produced rosette and test-tube-brush forms similar to those seen in smears of the original material. This organism was also described as pathogenic for rabbits. Its relationships and significance seem to be entirely unknown.

Leptothrix anaerobius tenuis (80) and *Actinomyces necrophorus* (77) are anaerobic gram-negative filamentous organisms which belong clearly with the genus *Bacteroides*.

Actinomyces muris (138) is a peculiar gram-negative organism of doubtful taxonomic position which, however, can hardly be placed with the true actinomyces without doing further violence to that already abused genus. This organism is the causative agent of one form of rat-bite fever and of other uncommon diseases of man. It was first described by Schottmüller in 1914 (123) under the name *Streptothrix muris ratti*, but has most commonly been called *Streptobacillus moniliformis* (79). The *Haverhillia multiformis* of Parker and Hudson (105) is evidently the same organism. Heilman (57) calls it *Asterococcus muris* and regards it as a bacteria-like phase of a member of the pleuropneumonia group. This group, of which the agent of bovine pleuropneumonia is the typal form, appears to lie parallel with the rickettsiae, but unrelated to them, in a position intermediate between the bacteria as a whole and the viruses. In this taxonomic position the organism would be separated from the actinomyces by the whole range of true bacteria.[2] It appears to inhabit the mouths of rats, mice and other animals, but has not been found in the human mouth, although similar forms have been observed in apparent symbiosis with *Bacteroides funduliformis* (33, 71, 34, 35).

THE BACTERIOLOGY OF ACTINOMYCOSIS

Actinomycosis may be defined as a subacute or chronic, usually progressive disease of cattle and other animals and of man, characterized by the development of indurated granulating swellings particularly in connective tissue, by suppuration usually of limited extent, and by the presence in the pus or lesions of *A. israeli*, demonstrable microscopically, culturally or both.

This is a restricted usage of the term actinomycosis. It follows the recommendations of Wright (153, 154), Colebrook (22) and others, and seems fully justified by available knowledge of the subject. It would exclude somewhat similar diseases in which the causative agent is (a) a saprophytic actinomycete (paractinomycosis, Colebrook 22); (b) *Actinobacillus ligniersi* (actinobacillosis,

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[2] Further data on *Streptobacillus moniliformis* are given in the following references: 68, 69, 70, 71, 33, 32, 119, 147.

Lignières and Spitz, 82, 83); (c) *Staphylococcus aureus* (botryomycosis, Magrou (91); and (d) the clinically distinct cutaneous mycetomas due to *Streptomyces madurae* and other saprophytic forms. In all these diseases club-bearing granules may be found in pus or in the tissues (see Shahan and Davis, 124). Some of them, particularly actinobacillosis,[3] appear to be considerably more common *in cattle* than true actinomycosis, so that diagnosis merely by demonstration of club-bearing granules is unjustified. *In man*, on the other hand, diseases other than true actinomycosis (excepting the cutaneous mycetomas), in which such granules are present, are so rare that demonstration of the granule, commonly accepted as diagnostic by pathologists, is hardly exceptionable.[4] Nevertheless, since clubs, or even the granules themselves, may be lacking in clinically typical instances of actinomycosis in man, which can nevertheless be diagnosed by microscopic demonstration of the branched gram-positive filaments, or better, by recovery of *A. israeli* in pure culture, the presence of club-bearing granules should not be made part of the definition of the disease.

A discussion of the clinical features, histopathology and therapy of actinomycosis is beyond the scope of this review. Colebrook (22), Wright (154) and Cope (25) present authoritative accounts of these aspects of the subject. The use of sulfonamide drugs in therapy has been described by Walker (146), Wilkinson (150), Cutting and Gebhardt (29), and Lyons, Owen and Ayers (87).

Experimental Actinomycosis. A. israeli, isolated either from actinomycosis or from the human mouth or throat, has been found capable of reproducing actinomycosis in experimental animals, and has been recovered in pure cultures from the lesions. Such experimental studies have nevertheless been far from decisive, and their results in the aggregate are difficult to evaluate. It has been shown repeatedly that pure cultures of *A. israeli* are at times capable of reproducing in animals lesions in which many of the features of the natural disease are duplicated, including the presence of granules and typical eosinophilic clubs. Yet the majority of such experiments have resulted negatively. Traumatization with a foreign body or by other means has not aggravated the effects of such inoculation, nor has the inclusion of other species of microörganisms in the inoculum. Single inoculations by any of several routes, in many species of animals, with or without traumatic or other manipulation, have resulted at most in the development of localized lesions without the wooden induration or the progressive and fatal character which often characterize the natural disease. Passage from animal to animal has not seemed to increase the virulence of the organism. Repeated inoculation by means apparently involving sensitization has yielded progressive and fatal actinomycosis of a seemingly typical charac-

[3] Further details on actinobacillosis are given in the following references: 82, 83, 107, 49, 15, 81, 90, 124.

[4] According to Cope (25) only 3 cases of actinobacillosis in man have been recorded. Drake, Sudler and Canuteson (39) have noted that 10 cases of botryomycosis in man have been reported. As noted previously, disease resembling true actinomycosis but due to a saprophytic actinomycete (Colebrook's "paractinomycosis") seems also to be very rare in man.

ter; yet again this result has been obtained without regularity. On the whole the data leave no doubt that *A. israeli* is the causative agent of actinomycosis; that potentially pathogenic actinomyces are widespread in the mouth and throat; and that secondary or accompanying infection is not necessary for the development of the disease. On the other hand the available data do not solve the problem of pathogenesis; they suggest that some condition or combination of conditions as yet unknown determines the occurrence of the disease, but fail to place the mechanism of its development beyond speculation.

Details of experimental actinomycosis may be described under the following heads:

Single inoculation of pure cultures of A. israeli. More or less extensive nodules or tumors, sometimes containing typical granules with clubs, more often containing recoverable actinomyces, have been produced in guinea pigs and rabbits by intraperitoneal inoculation (152, 153), and in rabbits by the intratesticular, subcutaneous or intracutaneous routes (86); in cattle by a single subcutaneous injection (81, 90), or by simultaneous multiple subcutaneous injections (100, 86); in sheep by the subcutaneous route (81); and in swine by inoculation into the testicle or the udder (90). Naeslund (100) also succeeded in infecting a single guinea pig, among several animals injected, by intraperitoneal inoculation. He and others have failed to enhance the virulence of the organism by passage of exudate or recovered cultures. His results and those of Lord and Trevett (86) are noteworthy in that the actinomyces used had been isolated from human tooth-scrapings rather than from actinomycosis. It must be emphasized that none of these workers obtained uniform results. Most of them, for example, failed to produce lesions in guinea pigs and rabbits; and Lignières (81) and Magnusson (90) in particular used a wide range of other animal species with negative or inconclusive results.

Single inoculation with trauma or other manipulation. Traumatization by various means has generally failed to increase the severity of experimental lesions or to yield a more uniform response than simple inoculation. Wright (153) was unconvinced that his positive findings in guinea-pigs and rabbits showed clear evidence of multiplication of the organism in the tissues, and attributed the somewhat more extensive lesions produced by Wolff and Israel (152) to the inclusion of agar in the inoculum. Magnusson (90), Naeslund (100), Emmons (41) and Sullivan and Goldsworthy (130) all obtained negative results or atypical lesions by inoculation of cultures and simultaneous implantation of a foreign body such as a horse-hair or a wood-splinter. Grootten (50), who failed to obtain lesions in rabbits after simple inoculation by several routes, succeeded in producing mesenteric nodules by the introduction of agar cultures of *A. israeli* through an incision in the abdominal wall. Lord and Trevett (86) used a similar method in guinea pigs with negative results. Negroni and Bonfiglioli (102) were able to produce indurated abscesses containing recoverable organisms, but apparently not containing granules or clubs, in several rabbits, a guinea pig and a white mouse, all by the subcutaneous route, but only when a suspension of sterile rabbit kidney was added to the inoculum.

Inoculation of unpurified source material containing A. israeli. Magnusson (90) reported that inoculation of animals with pus, derived from actinomycosis in cattle, and containing typical granules, yielded results similar to those obtained with pure cultures. Negroni and Bonfiglioli (102) failed to produce lesions in animals by inoculation of actinomycotic pus. Lord (85) inoculated guinea pigs intraperitoneally with scrapings from carious teeth or with material from tonsillar crypts, both of which had shown actinomyces in sections. In some instances, as would be expected (116) this treatment resulted in generalized peritonitis, probably fuso-spirochetal in character, in which actinomyces probably played no part. In more than half the animals, however, actinomycotic nodules and adhesions developed on the omentum after 2 to 6 weeks, and many of the lesions showed granules with eosin-staining clubs. Somewhat similar results are obtained by Naeslund (100) by intraperitoneal inoculation of guinea pigs with human saliva and tooth scrapings, but in a smaller proportion of animals. Subcutaneous inoculation of cattle with similar mixed material also produced small actinomycotic nodules in some instances. These findings fail to demonstrate that such unpurified inocula are more active in the production of actinomycotic lesions than are pure cultures.

Inoculation of cultures of A. israeli mixed with other bacteria. Pus from actinomycosis often contains contaminating bacteria, particularly when it is taken from a fistula draining to the skin; but there is no reason to believe that such accompanying bacteria need play any part in the natural pathogenesis of the disease. A small aerobic gram-negative organism called *Bacillus actinomycetum comitans* (*Actinobacillus actinomycetem-comitans*, Bergey *et al.* (5)) has been suggested as of special importance in this relation. Klinger (73) originally found this organism in four cases of actinomycosis, and Colebrook (21) found it around granules of *A. israeli* in 24 of 30 cases, but never independently. Pure cultures of the organism killed 2 of 3 rabbits with a quickly developing septicemia after intravenous injection of large doses. Bayne-Jones (4) has also recovered this organism from actinomycosis. Naeslund (100) and Sullivan and Goldsworthy (130) have reported, however, that mixtures of *B. actinomycetum comitans* with *A. israeli* were no more pathogenic for experimental animals than the actinomyces alone. These investigators and also Emmons (41) have also reported failure to produce significant lesions by inoculation of animals with cultures of other bacteria mixed with *A. israeli*.

Repeated inoculation of A. israeli. Progressive and fatal actinomycosis, evidently closely similar in some instances to the natural disease, has been produced in guinea pigs and rabbits by several investigators by repeated inoculation at intervals that might have permitted the development of allergy to the organism. Again, however, the results have been inconsistent and hardly more than suggestive. Mathieson, Harrison, Hammond and Henrici (92) gave as many as six injections of *A. israeli* to five guinea pigs at different intervals up to 46 days, using the intraperitoneal, intratesticular and subcutaneous routes. The animals did not respond to the first injections, but all reacted to later injections in varying degree with inconclusive indications of

actinomycosis. In all these animals, crushed granules from actinomycotic pus were used for some of the inoculations and pure cultures for the remainder. Four additional guinea pigs were inoculated only with pure cultures. One of these was given six injections at irregular intervals over a period of 3½ months, the first intratesticular, the second subcutaneous, and the others intraperitoneal. Another received only two injections, both intraperitoneal, at a 10-day interval. Neither animal showed more than slight local reactions grossly; but both, when killed 7 days after the last injection, showed apparently typical abdominal actinomycosis, with adhesions and multiple nodules containing clubbed granules. Findings in the other two animals, which received 3 and 5 injections respectively, were not characteristic.

These results could not be confirmed by Emmons (41), who gave repeated double inoculations (intraperitoneal and subcutaneous) of *A. israeli* to guinea pigs. As many as 8 such inoculations given at intervals of 4 days to 2 weeks elicited only small abscesses without progressive disease. Negative results were also reported in rabbits by Negroni and Bonfiglioli (102) after two injections of large doses of culture given at 11-day intervals by the intraperitoneal, intravenous or intratesticular routes.

The most striking findings following repeated inoculation of *A. israeli* have been those reported by Slack (126). Positive results were obtained by this worker accidentally following attempts to immunize rabbits with living cultures. The strain of *A. israeli* used had been isolated from pyorrheal pus. Large and increasing doses of a suspension in broth were injected intravenously at 3-week intervals. All of 4 rabbits given 3 or 4 such injections developed indications of chronic progressive disease, fatal in three; while the other was killed a few days after the fourth injection because of extreme emaciation and weakness. At autopsy all four animals were found to have actinomycotic lesions of the viscera, with clubbed granules in three. Similar results were obtained in a single guinea pig, which became emaciated and died after seven intraperitoneal injections of the same culture, given at 3-week intervals. Granules without clubs were found in the viscera of this animal.

Rosebury, Epps and Clark (115) have attempted to confirm the findings of Slack in a larger series of animals. Positive results were obtained with three out of nine strains of *A. israeli*, including two of three isolated from cervicofacial actinomycosis in man, and one of six isolated from gingival scrapings. A total of 24 guinea pigs and 16 rabbits were given from one to eight successive injections at 3-week intervals of massive doses of culture suspended in salt solution. Clear evidence of actinomycosis was obtained in only 5 animals, progressive and fatal in 2 guinea pigs and 1 rabbit, localized and benign in 2 other rabbits. Repeated injection by the intravenous or intraperitoneal routes seemed to be innocuous. Single or repeated subcutaneous injections usually produced only mild local lesions from which the organism could seldom be recovered. The fatal reactions were obtained by intrapleural injection, and by inclusion in the inoculum, given by this route or intravenously, of sterile pulverized salivary calculus. The organism injected was recovered from the lesions

of the fatal cases at autopsy. In the two guinea pigs with fatal thoracic actinomycosis granules without clubs were found in sections. One of the rabbits with localized infection, which showed only small nodules subcutaneously and in the lungs, all of which failed to yield actinomyces on culture, was the only animal in the series in which clubbed granules were found.

These results seem to emphasize the random and uncertain nature of the pathogenesis of actinomycosis. Repeated inoculation may induce generalized and fatal disease, whereas a single inoculation causes no more than localized lesions with little tendency to progress; yet in both instances the results on the whole are more often negative than positive. Determining conditions in the pathogenesis of progressive actinomycosis are evidently still unknown.

Attempts to determine whether allergy to *A. israeli* is related to the development of actinomycosis, by skin tests with living or killed cultures or culture filtrates, have given ambiguous results both in human subjects (92) and in rabbits (115). Healthy persons have been found to react irregularly, while patients with frank actinomycosis have failed to react in some instances. Inoculated rabbits reacted more strongly than uninoculated controls, but no clear difference was found between animals which later revealed no pathological changes and one animal that subsequently died with progressive actinomycosis. In man the reactivity of healthy subjects may be related to the occurrence of the organism as an indigenous parasite; and loss of sensitivity may possibly occur with the development of active disease, as is known to happen in tuberculosis (129).

Epidemiology. The theory of Bostroem (14) that actinomycosis is an exogenous infection derived by traumatization with grass, straw or grain which carries the infecting agent has been maintained by repetition in many text books and is still entertained, despite the almost universal failure to adduce evidence in support of it, and the opposite trend of nearly all the available data. Bostroem found vegetable particles in actinomycotic lesions in man and cattle; he was able, although very rarely, to isolate saprophytic actinomycetes from the diseased tissue; but he was unable to demonstrate their pathogenicity. As noted above, later workers have found that Bostroem's organism is seldom implicated in bovine actinomycosis and is even more rarely found in man. It has also been assumed that actinomycosis is an occupational disease of farmers and other agricultural workers, transmitted either by contact with infected animals or by direct traumatization with infected grain resulting, for example, from chewing straw. Davis (31) has reviewed this question and found that such rural groups seem no more subject to the disease than mechanics, clerks, or urban groups generally. Among 46 new cases described by Davis, only 15 were farmers and only 3 gave a history of chewing grass or straw. It has never been clearly shown, moreover, that actinomycosis can be communicated from animals to man, although many instances suggestive of such transmission have been recorded. Griffith (49) found in England that a high percentage of beef tongues and other tissues from animals slaughtered both locally and in Argentina contained club-bearing granules. Most of these

were identified as due to actinobacillosis, but in more than 5 per cent of instances they appeared to be true actinomycetes. Yet no instance of actinomycosis in man has ever been traced clearly to the ingestion of such contaminated food. Transmission from man to man seems to have been suggested in only two instances (2, 88) but without convincing evidence in either.

The causative agent of true actinomycosis, A. israeli, has never been found apart from a parasitic or pathogenic habitat; and its inability to grow at low temperatures, its lack of spores, and its requirement for reduced oxygen tension, all seem to make it incapable of existence under saprophytic conditions. It has been noted that potentially pathogenic A. israeli can frequently be found in the human mouth and throat in such local disorders as chronic tonsillitis, dental caries, gingivitis and pyorrhea, in the etiology of which these organisms appear to play no part. It is possible that they occur in small numbers in the fully healthy mouth; and that, in common with a large section of the mouth flora, they proliferate under a variety of different local conditions which contribute to or constitute the picture of poor oral hygiene. It seems justifiable, at all events, to include them with such microörganisms as the lactobacilli and the oral spirochetes (neither of which can be demonstrated constantly in the mouth) as members of the indigenous flora, and thus to assume that the oral and pharyngeal mucous membranes are their natural habitat. Since actinomyces isolated from such sources evidently belong in the same group as those obtained from clinical disease, and since strains from both sources have been found capable of producing experimental actinomycosis, the endogenous character of the natural disease seems inescapable. This view was first suggested by Wolff and Israel (152) and amplified by Wright (153). It has since come to be accepted virtually without dissent by all students of the parasitic actinomycetes. Naeslund (100) and others have suggested, moreover, that if trauma from straw, splinters and other vegetable matter plays a part in the pathogenesis of actinomycosis, it may be by facilitating the introduction of A. israeli into the tissues from a mucous membrane, or by aiding the growth of this organism in the tissues, rather than by causing exogenous infection with a saprophytic actinomycete.

Just how infection of adjacent or remote tissues develops through the agency of natural surface parasites nevertheless remains to be determined. It may be noted here that the comparative rarity of actinomycosis in man seems in line with the experimental data previously given in suggesting that the determining factor in the disease is something in addition to and less common than local trauma or even repeated autoinoculation, although both may well be contributory incidents in its pathogenesis. It may be significant that a history of tooth extraction or other injury to mouth or throat has frequently been obtained in cervico-facial actinomycosis (see Cope, 25; Davis, 31); that three instances of hand infection with actinomycosis following wounds inflicted by the human teeth have been recorded (89, 24, 112A); that pulmonary actinomycosis has been associated with aspiration of an extracted carious tooth or tooth fragment (61A, 148); and that an actinomycete has been recovered from the

blood immediately after extraction of a tooth (104). Finally, the apparent rôle of *A. israeli* in the deposition of salivary calculus, as detailed below, suggests that these deposits may play a part in the pathogenesis of actinomycosis. The experimental data of Rosebury, Epps and Clark (115) tend to support this view, but it has not been clearly demonstrated. This general subject invites further investigation, and currently improved methods for the cultivation and maintenance of *A. israeli*, as described above, may facilitate its solution.

Diagnosis. Diagnosis of actinomycosis in man rests on a combination of clinical signs and on microscopic demonstration, or preferably isolation, of the causative agent. Attempts to apply other specific diagnostic tests, such as the demonstration of agglutinins to *A. israeli* in the patient's serum (21) or of skin sensitivity to the organism or its products (92) have not been successful. Neuber (103) has recommended both a complement-fixation reaction and a skin test, but the conditions given for performing these tests make them appear impracticable. It is apparent, therefore, that no means of specific diagnosis is available in the absence of surface lesions, or where exudate cannot be obtained for bacteriological study.

Direct microscopic demonstration of *A. israeli* depends on the presence of gram-positive branched rods or filaments. Eosinophilic clubs are a confirmatory but not an indispensable adjunct. Isolation of *A. israeli* in pure culture, however, is the most convincing diagnostic procedure.

Where exudate containing typical granules can be obtained with reasonable expectation that contaminating bacteria are absent, inoculation of glucose-agar shake cultures may suffice for isolation. The granule is transferred to a tube of melted agar that has been cooled to about 45°C, broken against the wall of the tube, and distributed through the medium. Several additional tubes of melted glucose agar should then be inoculated serially. These cultures may be incubated at 37°C in air. After 3 to 6 days, successful uncontaminated cultures show the characteristic whitish spherical or mulberry-like colonies growing in the depths of the agar, often with a dense zone of colonies about a centimeter below the free surface. The diagnosis is confirmed by demonstration in such a colony, removed with a capillary pipette, of a branched mycelium or of branched twig-like gram-positive rods and short filaments.

The method used by Rosebury, Epps and Clark (115), although not so simple as the preceding, is recommended as preferable, and is almost indispensable if the source material is contaminated. For this purpose Bacto brain-heart medium containing 2 per cent of agar is used in streaked plates. The granule, or a loopful of exudate or other source material, without washing or other manipulation, is streaked serially on four plates of this medium with a bent glass rod. A loopful of sterile broth may be used on each plate to help moisten and distribute the inoculum. The plates are incubated for 4 to 6 days at 37°C in an anaerobic jar with hydrogen, catalyzed by heated platinum or palladium, and containing about 5 per cent of carbon dioxide. Typical colonies may then

be found and identified as described previously.[5]   A typical colony should be fished to a glucose-agar shake tube and the subculture used for confirmation as described above.

Prevention. Since we are ignorant of the mechanism whereby *A. israeli* proceeds from its habitat on the mucous membranes to set up progressive lesions in the deeper tissues, no specific recommendations for prevention can be given.   In general the data seem to constitute one reason among others for the avoidance of excessive trauma in surgery of the mouth and pharynx.   In view of the indications that the organism is found particularly in the presence of local inflammatory processes like pyorrhea or gingivitis, the prevention or control of these disorders may be expected to help prevent actinomycosis, among other possible systemic sequels of defective oral hygiene.

<center>SALIVARY CALCULUS</center>

There is convincing evidence that microörganisms play an important part in the deposition of salivary calculus or tartar,—the calcified masses that form on the surfaces of teeth or other fixed structures (such as prosthetic appliances) in the mouth.   Much of the evidence points to *Actinomyces israeli* as the agent in the formation of these concretions; but the data leave many questions unanswered, particularly with reference to the mechanism whereby the deposits are formed.[6]

The several clinical varieties of tartar—supragingival and subgingival calculus and salivary duct stones—all appear to be similar in chemical composition (74) and in microscopic structure (98, 99), as well as in their content of microorganisms (99, 8).   They all seem to originate from saliva.   Physical differences between concretions from the different sites, in hardness, texture and color, are probably dependent on the location in which the mass is deposited (75). *Supragingival calculus*, the variety that forms in largest amount and is most readily obtained for study, is deposited on exposed surfaces of the teeth, preferentially on those adjacent to the orifices of the salivary ducts; i.e., on the lingual surfaces of the lower anterior teeth and on the buccal surfaces of the upper first and second molars.   It is also found frequently on malposed or irregular tooth surfaces elsewhere, in areas that are difficult to reach or are habitually not reached by the toothbrush (59), and on the surfaces of teeth that are not used in chewing because of pain or because opposing teeth are lacking.   When freshly deposited these concretions are cream-colored or yellowish and soft, so that they may easily be brushed away (13); but they become hard and firmly adherent within a few days, and later they become stained by food or tobacco.   *Subgingival calculus*, sometimes miscalled "serumal" calculus, is

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[5] This method has been found successful for isolation of *A. israeli* both from pus from actinomycosis and from grossly contaminated sources such as gingival scrapings.   It should be noted that in view of the sensitivity of the method and of the frequent occurrence of the organism in the mouth and throat, the recovery of *A. israeli* from sputum or other material contaminated from these areas is not necessarily diagnostic of actinomycosis.

[6] For general reviews of salivary calculus see Rosebury and Karshan (117) and Tenenbaum and Karshan (136).

deposited on the tooth surface under the free margin of the gum within the confines of the gingival crevice or in a pyorrheal pocket. It is generally harder than the supragingival form, perhaps because it is formed more slowly, and is usually deeply stained, probably as a result of small hemorrhages around it. This form of tartar is found characteristically in pyorrheal pockets, but it is not known whether it is a cause or an effect of the pocket. Supragingival calculus may cause irritation of the underlying epithelium, but because of its position on the outer gingival epithelium it probably has no bearing on pocket formation. That both varieties may be irritating, however, and may aggravate gingival and periodontal lesions if they do not cause them, is indicated by the beneficial results that generally follow their removal.

The inorganic composition of tartar and duct stones has been studied by several investigators (131, 74, 108, 64, 45) with a good agreement in the data which, especially in view of differences in analytical methods, suggests that the concretions originate by a uniform process. Mineral matter constitutes about 70 to 80 per cent of the mass, and consists mainly of calcium and phosphorus, with magnesium and other elements in smaller amounts. The proportions of calcium, phosphorus and magnesium in tartar are similar to those in dentin, and appear to be present either as tricalcium phosphate or as a hydroxyapatite or other apatite-like salt (64, 106, 93). Chemical study of the organic portion of subgingival tartar (45) has indicated about 8.3 per cent of protein, consisting of keratin, mucin and nucleoprotein; and 2.7 per cent of fatty material. The keratin and mucin appear to be derived by inclusion in the mass of desquamated epithelial cells and of saliva, respectively.

Microörganisms in Calculus. All three clinical varieties of calculus, when studied in carefully decalcified sections stained by Gram's method, have been found to contain a characteristic stroma of filamentous microörganisms, in parts of which true branching can be seen (98, 99, 128, 51, 52, 53, 54, 133). According to Naeslund, this stroma can be demonstrated in all specimens of tartar if careful histological methods are used. Branching filaments are seen particularly in the deeper or older portions of the mass, while continuous with these in the more peripheral or newer portion the stroma may consist of unbranched filaments in palisade or radial arrangement. In some instances, however, only branching filaments can be seen, and these pervade the entire mass. Naeslund reported that typical actinomycotic clubs may be demonstrable occasionally in tartar, especially in the subgingival variety.

Bacteriological studies of tartar always yield a variety of indigenous oral microörganisms, as would be expected. It seems significant, however, that *Actinomyces israeli* has been isolated both from the surface and from the depths of tartar more often than other filamentous microörganisms, and that it appears to be the only organism among those commonly recovered whose morphology would account for the characteristic branched stroma seen in sections. Organisms whose properties as they were described indicate their identity with *A. israeli* have been isolated from tartar by Bulleid (18, 19), Naeslund (99), Bibby and Knighton (10, 11); Grythe (52, 53, 54), and Rosebury, Epps and Clark

(115). Naeslund, whose findings seem particularly noteworthy in view of his earlier (97) careful studies of actinomycetes, was able to isolate anaerobic actinomyces repeatedly from both supragingival and subgingival concretions, more often from the peripheral calcified portion than from the deeper portion. He noted that such organisms could be recovered much more commonly than the unbranched filamentous forms. He and others have called such unbranched forms *Leptothrix* or *Leptotrichia*. As noted above, organisms from tartar which have been thus named cannot be clearly identified, and they appear to play no important part in the formation of the deposits. An exception is the organism isolated by Bulleid (18, 19). Although he called it *Leptothrix buccalis*, Bulleid's description of a gram-positive form showing true branching, preferring anaerobic conditions, and growing as small adherent raised rough colonies, suggests that he was dealing with *A. israeli*.

That microörganisms play an essential part in the deposition of tartar is indicated by the findings of several investigators, who were able to produce deposits similar to natural tartar *in vitro*. Such artificial deposits have appeared in stagnating saliva (108, 99, 55); in artificial mixtures of salts and protein made to resemble saliva and left exposed to air and contamination (108); and in culture media containing calcium salts and inoculated with pure cultures of actinomycetes or other organisms (18, 19, 99, 8). In the latter experiments artificial tartar deposition did not occur in sterile media, and was inhibited by the addition of disinfectants or when killed instead of viable organisms were inoculated. As noted above, the organism studied by Bulleid appears to have been *A. israeli*. Naeslund also produced concretions with pure cultures of actinomyces, and apparently succeeded likewise with his unidentified "leptothrix." Bibby reported best results with unidentified filamentous organisms and with *Proteus sp.* and *Bacillus subtilis*. The data of Bulleid and Naeslund suggest the capacity of *A. israeli* to form concretions, but the evidence as a whole leaves little doubt that other organisms may be equally capable of forming them. It seems noteworthy that *A. israeli* is not conspicuous for its calcifying action in most other areas. The "sulfur granules" of actinomycosis are only rarely calcified (81). Cornell (26) has described a case of actinomycosis of the internal female genitalia in which the granules, found in sections, seemed to have been composed largely of unidentified crystals. Elliot (40) noted the occurrence of calcified sulfur granules in actinomycosis of the lachrymal canaliculi. Of special interest in this connection is the view of Söderlund (128) that salivary duct stones are formed as a result of a localized actinomycotic infection of the ducts. Precipitation of calcium salts by *A. israeli*, although by no means a constant phenomenon, may be conditioned by the environment in which the organism grows.

Mechanism of Tartar Deposition. The manner in which calculus is deposited, whether by the action of *A. israeli* or otherwise, has been the subject of much speculation and experiment but has thus far remained obscure. The older theories of tartar formation have been discussed by Rosebury and Karshan (117), and may be reviewed here briefly. The belief that loss of carbon dioxide

from saliva is the essential cause of tartar formation (96, 67) is invalidated by the findings (a) that the change in CO_2 content of saliva on exposure to air is insignificant despite loss of the gas (20), and (b) that limitation of the escape of CO_2 from stagnating saliva *in vitro* has not affected the formation of artificial deposits (55, 99). The theory of Prinz (108) which suggests that precipitation depends on loss of protective colloid as a result of surface concentration of colloids in stagnating saliva fails to take account of bacterial action, as disclosed by subsequent studies, and rests on the doubtful assumption that salivary stagnation is essential to the process. It seems hardly likely that the oral regions nearest the salivary duct orifices, in which tartar forms by preference, afford better conditions for stagnation than other parts of the mouth. Broderick's view (16) that tartar deposition depends upon a generalized alkalosis which is reflected in increased salivary alkalinity has received little support from the data of Tenenbaum and Karshan (134, 135, 136), who found the difference in pH between calculus-free and calculus-forming groups of persons to be small and of uncertain significance in stimulated saliva, while the average pH values in unstimulated saliva for the two groups were nearly identical. Changes in the pH of the medium, moreover, have not been found important in the deposition of artificial concretions (55, 8).

Naeslund (99) has suggested that calculus is formed through the activity of actinomyces and "leptothrix," which induce the precipitation of salivary salts and also act as a matrix both to retain the deposit and to attach it to the tooth surface. Since the organisms remain alive at the periphery of the calcified mass, the process would be progressive rather than self-limiting. This part of Naeslund's theory—except for its inclusion of the ambiguous "leptothrix"—is in good agreement with the facts presented above. The known ability of *A. israeli* to form a dense branched mycelium which tends to attach itself firmly to the surface on which it grows may, indeed, make this organism uniquely capable of acting as a matrix for the deposition of tartar.

Naeslund goes further, however, to suggest that the mechanism whereby these microörganisms precipitate salivary salts depends, first, on the loss of salivary colloids, and hence of their "protective effect" on the salts in solution, as a result of bacterial proteolysis; and secondly, on a consequent or correlated increase in the pH of the medium. As noted previously, Naeslund (97) reported that some strains of *A. israeli* were proteolytic and could induce an alkaline reaction; but other workers have uniformly failed to confirm this finding. Naeslund himself (99) found that the proteolytic action of his "leptothrix" was "weak, in some cases uncertain," and stated that "on account of the scanty growth no definite change in reaction could be detected" with this organism. There is no doubt that the mechanism postulated by Naeslund is theoretically sound, in that the changes he suggested would result in the precipitation of salivary calcium salts; but the data do not make it clear that the microorganisms concerned are capable of inducing them.

An alternative explanation, involving the action of phosphatases, may perhaps be substituted for the doubtful part of Naeslund's theory. The evidence

on this point, however, is incomplete and hardly more than suggestive. The action of these enzymes, which liberate phosphate from organic combination and are known to take part in the deposition of the calcified tissues, was first suggested as important in tartar formation by Adamson (1) and Smith (127). These workers believed that phosphatase derived from the gingival tissue rather than from microörganisms is the active agent in the process. Zander (155), however, has shown by a histochemical method that phosphatase is not present in gingival epithelium, although it is found in the connective tissue, and particularly in capillary endothelium. Phosphatase would thus be liberated from the gums only under pathological conditions. On the other hand it is known that microörganisms may produce phosphatase. Glock, Murray and Pincus (46) found it produced by several oral species, including strains of actinomycetes which, however, appear to have been saprophytes rather than *A. israeli*. Smith (127) had shown that phosphatase activity in saliva was concentrated in the sediment after centrifugation; and, as Zander (155) has pointed out, this finding may indicate that the greater part of the activity in saliva is derived from the oral microörganisms. The phosphatase activity of many bacteria may explain their apparent ability to produce concretions *in vitro*, as noted above. Yet it has not been shown that *A. israeli* produces phosphatase, and no direct evidence has been provided to link these enzymes to tartar formation. Saltzmann (121) and Tascher and Wagreich (132) have both found that the degree of salivary phosphatase activity is not related to the presence or characteristic absence of tartar on the teeth. The enzyme that may be associated with tartar formation, however, would be expected to constitute no more than a small fraction of the total phosphatase content of saliva at large; hence the negative import of these results may not be significant.

Smith (127) and Glock, Murray and Pincus (46) found maximal phosphatase activity in saliva in the pH range 5.0 to 6.0; while Tascher and Wagreich (132) have reported phosphatase activity both at pH 4 and at pH 7.4 in a sample of supragingival calculus. The latter workers also observed that human parotid saliva collected directly from the orifice of Stenson's duct showed phosphatase activity. Although their experiment does not necessarily exclude microörganisms as the source of the enzyme, it seems improbable that salivary phosphatase is derived entirely from the microörganisms of the mouth.

If *Actinomyces israeli* is the active agent in the deposition of calculus, its capacity to form tartar would seem to depend chiefly on its morphology and characteristic growth which, as Naeslund suggested, would attach the growing mass to the tooth surface and serve as a stroma or matrix for continued formation of the deposit. Being unique in this respect, it might not need to be unique in possessing a capacity to precipitate calcium salts, whatever the mechanism of such precipitation may be, in order to serve as the only agent in which all the necessary properties for calculus deposition are combined. A clear demonstration of the capacity of *A. israeli* to form tartar, and of the means it employs to do so, is nevertheless needed to make this suggestion something more than the best guess afforded by the available information.

Predisposing Factors in Tartar Formation. Salivary calculus is seldom found in the mouths of children, but its occurrence seems to increase with advancing age, and it is very prevalent among adults. It has been found on the teeth of Egyptian mummies, Incas and other ancient and aboriginal peoples (66), as well as on those of modern primitives such as Eskimos (118) and other isolated groups such as the natives of Tristan da Cunha (122, 44). Such groups are known to be comparatively free from dental caries; and it is a matter of common observation that mouths with particularly heavy tartar deposits may be found free from decay. Rosebury and Waugh (118) reported that among a group of Alaskan Eskimos those with active caries generally had less tartar and those free from caries generally had more tartar than would have been expected on the basis of their average age alone. It is of interest, moreover, that groups of persons who tend habitually to form tartar in large amounts have been found by Tenenbaum and Karshan (134, 135, 136) to have significantly higher levels of total calcium and inorganic phosphorus, in both stimulated and unstimulated saliva, than groups lacking this tendency. Similar findings have been reported for Alaskan Eskimos (63, 65) and for Greenland Eskimos (62). The differences were evidently not due to the presence of large calcified masses in the mouths of the group with tartar, since similar values were obtained before and after removal of the deposits (135). The protein content of saliva was not found significantly different in the two groups. In stimulated saliva the average pH was slightly lower for the calculus-free group, the difference between the two groups being of uncertain significance; while the average values for pH of unstimulated saliva from the two groups were nearly identical.

Willsmore (151) has reported that the occurrence of calculus is related to oral hygiene, indicated in terms of the frequency of tooth brushing, and also to variation in tendency of saliva to deposit cellular débris on standing. Among 36 persons who brushed their teeth at least twice a day, and whose saliva did not tend to deposit débris, none had calculus. At the other extreme, among 51 persons who never used the toothbrush, and whose saliva tended markedly to deposit débris, all but one had calculus, and 45 had abundant deposits. Data for groups with intermediate degrees of these two conditions were generally consistent with this pattern.

Since tartar is soft and easily removed when it is first formed, the development of hard deposits can presumably be prevented by regular and efficient brushing of the teeth. Otherwise, however, it appears that a tendency to form calculus may depend on individual variation in the composition of saliva. There are no data on the incidence of *A. israeli* in the mouth correlated with such tendency. It has been noted that persons with tartar generally have a higher salivary concentration of the principal inorganic constituents of the deposit. This finding may imply that such higher concentrations are required in order that the mechanism of precipitation may become operative.

Tartar in itself (excluding duct stones), in view of its widespread occurrence, can hardly be looked upon as pathological; but on clinical grounds it seems

clear that it may lead to or aggravate pathological changes in the adjacent soft tissues. The occurrence of viable *Actinomyces israeli* in tartar has led to the suggestion (23) that detached masses of this material may be instrumental in the pathogenesis of actinomycosis, whether by aspiration into the respiratory tract or by other means; and as noted previously suggestive evidence in support of this view has been provided from experimental studies (115). It is evident, however, that only further research can clarify the many questions in this field that remain unanswered.

SUMMARY

The name *Actinomyces israeli* is applied in this review in accordance with priority and usage to those gram-positive, branched microörganisms which are generally cultivable under partial anaerobiosis, which are parasitic on certain mucous membranes, and which are responsible for true actinomycosis in man and animals. The morphology of this organism is described as it appears in sections of and in exudates from the lesions of actinomycosis, and also in preparations from the human mouth and throat, and in cultures. Methods are given for its cultivation and maintenance; and its cultural characteristics are described under various conditions of growth. It is noted that *A. israeli* is a heterogeneous species, but that attempts to subdivide it have not as yet given satisfactory results. Interrelationships are considered of *A. israeli* with bacteria and fungi in general, with the anaerobic lactobacilli, and with the saprophytic actinomycetes.

Leptotrichia buccalis, an unbranched gram-positive parasite of the human mouth, is described, and data are reviewed which suggest that it has no pathogenic significance. Other microörganisms, described as leptotrichia, actinomycetes, or under other names, are considered in an effort to place them taxonomically. These include *Leptotrichia* (*Cladothrix*) *placoides*, *Leptothrix racemosa* and *L. falciformis*, and *Actinomyces muris*.

Actinomycosis is treated with special reference to the experimental production of the disease in animals. Data are given which indicate that single inoculations of pure cultures of *A. israeli* have yielded non-progressive lesions irregularly; trauma or other manipulation with such single inoculations has not seemed to aggravate this effect; nor has the use for inoculation of pus from naturally occurring actinomycosis, or of other unpurified material containing the causative organism. Supplementing pure cultures of *A. israeli* with *Bacillus actinomycetum comitans* or other bacteria has likewise failed to enhance the infectivity of the inoculum. On the other hand, progressive and fatal actinomycosis has been produced experimentally, in rabbits and guinea pigs by repeated injection of *A. israeli*. Here again, however, the results have been irregular and suggest that essential factors in the pathogenesis of actinomycosis are still unknown. It has not been clearly demonstrated that allergy to *A. israeli* is such a factor. The common occurrence of potentially pathogenic *A. israeli* on the oral and pharyngeal mucous membranes in the absence of actinomycosis, the apparently strictly parasitic habit of the organism, and most of the avail-

able epidemiological data all point to an endogenous origin of the natural disease.

Methods for the bacteriological diagnosis of actinomycosis are given, and the problem of prevention of the disease is considered briefly.

The data reviewed on the nature, composition and manner of formation of salivary calculus or tartar indicate that these concretions are precipitated from saliva through the action of oral microörganisms. Suggestive but incomplete evidence points to *Actinomyces israeli* as the causative agent. The mechanism of tartar deposition, although the subject of much speculation, has not been clarified. The hypothesis that seems most promising involves the action of phosphatase of microbial origin. It is suggested that the capacity of *A. israeli* to grow as a branched mycelium attached to tooth surfaces, by providing a stroma for tartar deposition, may make this organism peculiarly capable of forming calculus. The saliva of persons who tend to form tartar has been found to contain higher average concentrations of calcium and inorganic phosphorus than the saliva of non-formers, and it is possible that such high levels may be required for their precipitation.

BIBLIOGRAPHY

(1) ADAMSON, K. T. 1929 The rôle of enzyme action in the formation of dental calculi. Australian J. Exptl. Biol. Med. Sci., **6**, 215–227.

(2) BARACZ, R. V. 1889 Übertragbarkeit der Aktinomykose vom Menschen auf den Menschen. Wien. med. Presse, **30**, 6–11.

(3) BARTELS, H. A. 1943 A filamentous microörganism isolated from stained teeth. J. Dental Research, **22**, 97–102.

(4) BAYNE-JONES, S. 1925 Club-formation by *Actinomyces hominis* in glucose broth, with a note on *B. actinomycetum-comitans*. J. Bact., **10**, 569–575.

(5) BERGEY, D. H., et al. 1939 Bergey's Manual of Determinative Bacteriology. Williams & Wilkens Co., Baltimore, 5th ed.

(6) BEUST, T. 1908 A contribution of the morphology of the microörganisms of the mouth. Dental Cosmos, **50**, 594–595.

(7) BEUST, T. B. 1937 Morphology of the fusiform bacillus. J. Dental Research, **16**, 379–386.

(8) BIBBY, B. G. 1935 The formation of salivary calculus. Dental Cosmos, **77**, 668–676.

(9) BIBBY, B. G., AND BERRY, G. P. 1939 A cultural study of filamentous bacteria obtained from the human mouth. J. Bact., **38**, 263–274.

(10) BIBBY, B. G., AND KNIGHTON, H. T. 1935 Mouth organisms resembling actinomyces. J. Dental Research, **15**, 171.

(11) BIBBY, B. G., AND KNIGHTON, H. T. 1941 The actinomyces of the human mouth. J. Infectious Diseases, **69**, 148–154.

(12) BIGGART, J. H. 1934 Actinomycosis graminis. Bull. Johns Hopkins Hospital **54**, 165–173.

(13) BLACK, G. V. 1911 Beginnings of pyorrhoea alveolaris treatment for prevention. Dental Items of Interest, **33**, 420–455.

(14) BOSTROEM, E. 1891 Untersuchungen über die Aktinomykose des Menschen. Beitr. path. Anat., **9**, 1–240.

(15) BOSWORTH, T. J. 1923 The causal organism of bovine actinomycosis. J. Comp. Path. Therap., **36**, 1–22.

(16) BRODERICK, F. W. 1936 Principles of Dental Medicine, the Medical Aspects of Dental Disease. C. V. Mosby, St. Louis, 2nd ed.

(17) BUEHLMANN, F. 1840 Über eine eigenthümliche, auf den Zähnen des Menschen vorkommende Substanz. Arch. Anat., Physiol. wiss. Med., 442–445.

(18) BULLEID, A. 1924 An experimental study of *Leptothrix buccalis*. Guy's Hosp. Rept., **74**, 444–458.

(19) BULLEID, A. 1925 An experimental study of *Leptothrix buccalis*. Brit. Dental J., **46**, 289–300.

(20) CLARK, C. W., AND CARTER, K. L. 1927 Factors involved in the reaction changes of human saliva. J. Biol. Chem., **73**, 391–404.

(21) COLEBROOK, L. 1920 The mycelial and other micro-organisms associated with human actinomycosis. Brit. J. Exptl. Path., **1,**, 197–212.

(22) COLEBROOK, L. 1921 A report upon 25 cases of actinomycosis, with especial reference to vaccine therapy. Lancet **200**, 893–899.

(23) COLEBROOK. L. 1931 The anaerobic actinomyces. A system of Bacteriology in Relation to Medicine. Medical Research Council, London, **8**, 78–86.

(24) COPE, V. Z. 1915 A clinical study of actinomycosis, with illustrative cases. Brit. J. Surg., **3**, 55–81.

(25) COPE, Z. 1938 Actinomycosis. Oxford University Press, London.

(26) CORNELL, V. H. 1934 Actinomycosis of tubes and ovaries. Am. J. Path., **10**, 519–530.

(27) CRECELIUS, H. G. AND RETTGER, L. F. 1943 The intestinal flora of the guinea pig. J. Bact., **46**, 1–13.

(28) CROWLEY, M. C. 1941 The isolation of an actinomycetes-like organism from root canals. J. Dental Research, **20**, 189–194.

(29) CUTTING, W. C., AND GEBHARDT, L. P. 1941 Inhibitory effects of sulfonamides on cultures of *Actinomyces hominis*. Science, **94**, 568–569.

(30) DAVIS, D. J. 1914 The actinomyces-like granules in tonsils. J. Infectious diseases, **14**, 144–158.

(31) DAVIS, M. I. J. 1941 Analysis of forty-six cases of actinomycosis with special reference to its etiology. Am. J. Surg., **52**, 447–454.

(32) DAWSON, M. H., AND HOBBY, G. L. 1939 Rat-bite fever. Trans. Assoc. Am. Physicians, **54**, 329–332.

(33) DIENES, L. 1939 L type variant forms in cultures of various bacteria. Proc. Soc. Exptl. Biol. Med., **42**, 636–640.

(34) DIENES, L. 1942 The significance of the large bodies and the development of L type of colonies in bacterial cultures. J. Bact., **44**, 37–73.

(35) DIENES, L., AND SMITH, W. E. 1942 Relationship of pleuropneumonia-like (L) organisms to infections of human genital tract. Proc. Soc. Exptl. Biol. Med., **50**, 99–101.

(36) DOBELL, C. 1932 Antony van Leewenhoek and his "little animals". Harcourt, Brace & Co., New York.

(37) VON DOBRZYNIECKI, A. R. 1937 Zwei chromogene Mikroorganismen der Mundhöhle. Zentr. Bakt. Parasitenk. Abt. I, **21**, 833–835.

(38) VON DOBRZYNIECKI, A. R. 1898 Beitrage zu Bakteriologie der Zahncaries. Zentr. Bakt., Abt. I, **23**, 976–979.

(39) DRAKE, C. H., SUDLER, M. T., AND CANUTESON, R. I. 1943 A case of staphylococci actinophytosis (bothyomycosis) in man. The tenth reported human case. J. Am. Med. Assoc., **123**, 339–341.

(40 ELLIOT, A. J. 1941 Streptothricosis of the lacrimal canaliculi—Report of 9 cases. Am. J. Ophthalmol., **24**, 682.

(41) EMMONS, C. W. 1938 The isolation of *Actinomyces bovis* from tonsillar granules. U. S. Pub. Health Repts., **53**, 1967–1975.

(42) ERIKSON, D. 1935 The pathogenic aerobic organisms of the actinomyces group. Med. Research Council (Brit.) Special Rept. Series No. 203.

(43) ERIKSON, D. 1940 Pathogenic anaerobic organisms of the actinomyces group. Med. Research Council (Brit.), Special Rept. Series No. 240.

(44) FERGUSON, R. A. 1934 A dental survey of the school children of American Samoa. J. Am. Dental Assoc., **21**, 534–549.

(45) GLOCK, G. E., AND MURRAY, M. M. 1938 Chemical investigation of salivary calculus. J. Dental Research, **17**, 257–264.

(46) GLOCK, G. E., MURRAY, M. M., AND PINCUS, P. 1938 The origin and significance of salivary phosphatase. Biochem. J., **32**, 2096–2104.

(47) GOADBY, K. W. 1903 The Mycology of the Mouth. Longmans, Green & Co., London.

(48) GOODRICH, H. P., AND MOSELEY, M. 1916 On certain parasites of the mouth in cases of pyorrhea (preliminary communication. J. Roy. Microscop. Soc., 513–527.

(49) GRIFFITH, F. 1916 On the pathology of bovine actinomycosis. J. Hyg., **15**, 195–207.

(50) GROOTEN, O. 1934 Caractéres généraux et pouvoir pathogène expérimental de l'*Actinomyces israëli*. Ann. inst. Pasteur, **53**, 311–323.

(51) GRYTHE, O. 1933 Spiller visse sopparter en rolle for tannstersdannelse. Norske Tannlaegeforenings Tidende, **43**, 1–6.

(52) GRYTHE, O. 1938 Leptotrix. Experimentelle Untersuchungen an Leptotrix die aus klinisch gesunden Zahnfleisch gezüchtet wurden. Fabritius & Sønner, Oslo.

(53) GRYTHE, O. 1938 The rôle of Actinomyces and Leptothrix as an etiological factor in the formation of tartar and salivary calculi. Norske Tannlaegiforenings Tidende, **48**, 433–447.

(54) GRYTHE, O. 1938 Leptothrix. Experimentelle Untersuchungen an Leptothrix die aus Klinisch gesunden Zahnflesich gezüchtet wurden. Norske Tannlaegeforenings Tidende, **48**, Suppl. No. 5.

(55) HALL, I. C., AND WESTBAY, C. 1925 Bacterial factors in pyorrhea alveolaris. (II) Further studies on reaction changes in saliva, due to microörganisms, and their possible relation to the formation of dental calculus. Dental Cosmos, **67**, 115–124.

(56) HARZ, IN BOLLINGER, O. 1877 Ueber eine neue Pilzkrankheit beim Rinde. Zentr. med. Wiss., **15**, 481–485.

(57) HEILMAN, F. R. 1941 A study of *Asterococcus muris* (*Streptobacillus moniliformis*) 1. morphologic aspects and nomenclature. J. Infectious Diseases, **69**, 32–44.

(58) HENRICI, A. T. 1930 Molds, Yeasts and Actinomycetes. John Wiley & Sons, New York.

(59) HIRSCHFELD, I. 1939 The Toothbrush: Its Use and Abuse. Dental Items of Interest Publishing Co., Brooklyn, N. Y.

(60) HOLM, P. 1930 Comparative studies on some pathogenic anaërobic actinomyces. Acta Path. Microbiol. Scand., Suppl. 3, 151–156.

(61) ISRAEL, J. 1878 Neue Beobachtungen auf dem Gebiete der Mykosen des Menschen. Virchow's Arch., **74**, 15–53.

(61A) ISRAEL, J. 1886 Ein Beitrag zur Pathogenese der Lungenaktinomykose. Verhandl. deut. Ges. Chir. **15**, part 2, 36–40.

(62) KARSHAN, M., PEDERSEN, P. O., SIEGEL, E. H., AND TENENBAUM, B. 1940 Biochemical studies of the saliva of Greenlanders correlated with dental caries and the occurrence of salivary calculus. J. Dental Research, **19**, 303–304.

(63) KARSHAN, M., ROSEBURY, T., AND WAUGH, L. M. 1939 Dental caries among Eskimos of the Kuskokwim area of Alaska. II. Biochemical characteristics of stimulated saliva correlated with dental caries and occurrence of salivary calculus. Am. J. Diseases Children, **57**, 1026–1034.

(64) KARSHAN, M., AND SCHROFF, J. 1928 Composition of some salivary calculi. J. Dental Research, **8**, 454–455.

(65) KARSHAN, M., SIEGEL, E. H., AND WAUGH, L. M. 1940 Biochemical studies of the saliva of Eskimos correlated with dental caries and the occurrence of salivary calculus. Am. J. Diseases Children, **59**, 39–44.

(66) KELSEY, C. J. 1914 Dental calculus. Brit. Dental J., **35**, 810–817.

(67) KIRK, E. C. 1911 American Textbook of Operative Dentistry. Lea & Febiger, Philadelphia, 4th ed.

(68) KLIENEBERGER, E. 1935 The natural occurrence of pleuropneumonia-like organisms in apparent symbiosis with *Streptobacillus moniliformis* and other bacteria. J. Path. Bact., **40**, 93–105.

(69) KLIENEBERGER, E. 1936 Further studies on *Streptobacillus moniliformis* and its symbiont. J. Path. Bact., **42**, 587–598.

(70) KLIENEBERGER, E. 1938 Pleuropneumonia-like organisms of diverse provenance: some results of an enquiry into methods of differentiation. J. Hyg., **38**, 458–476.

(71) KLIENEBERGER, E. 1940 The pleuropneumonia-like organisms: further comparative studies and a descriptive account of recently discovered types. J. Hyg., **40**, 204–222.

(72) KLIGLER, I. J. 1915 A biochemical study and differentiation of oral bacteria with special reference to dental caries. J. Allied Dental Soc., **10**, 141–166; 282–330; 445–458.

(73) KLINGER, R. 1912 Untersuchungen über menschliche Aktinomykose. Zentr. Bakt. Parasitenk., Abt. I, Orig., **62**, 191–200.

(74) KRANZ, P. 1919 Betrachtungen über den Zahnstein. Deut. monatschr. Zahnh., **37**, 8–18.

(75) KRONFELD, R. 1933 Histopathology of the Teeth. Lea & Febiger, Philadelphia.

(76) KÜTSING, F. T. 1843 *Phycologia generalis* oder Anatomie, Physiologie und Systemkunde der Tange. F. A. Brockhaus, Leipzig.

(77) LEHMANN, K. B., AND NEUMANN, R. 1899 Atlas und Grundriss der Bakteriologie und Lehrbuch der speciellen bakteriologischen Diagnostik. J. F. Lehmann, Munchen, a. Aufl., Part 2.

(78) LENTZE, F. A. 1938 Zur Bakteriologie und Vakzinetherapie der Aktinomykose. Zentr. Bakt. Parasitenk., Abt. I, **141**, 21–36.

(79) LEVADITI, C., NICOLAU, S., ET POINCLOUX, P. 1925 Sur le rôle étiologique de *Streptobacillus moniliformis* (nov. spec.) dans l'érythème polymorphe aigu septicémique. Compt. rend., **180**, 1188–1190.

(80) LEWKOWICZ, X. 1901 Recherches sur la flore microbienne de la bouche des nourrissons. Arch. med. exptl. anat. path., **13**, 633–660.

(81) LIGNIÈRES, J. 1924 Nouvelle contribution à l'étude des champignons produisant les actinomycoses. Ann. parasitol. humaine et comparée, **2**, 1–25.

(82) LIGNIÈRES, J., ET SPITZ, G. 1902 Actinobacillose. Rev. soc. med. argentina, **10**, 5–114.

(83) LIGNIÈRES, J., ET SPITZ, G. 1902 Actinomycosis. Bull. Soc. centr. Med. Vetr., Paris, **20**, 487, 546.

(84) LORD, F. T. 1910 A contribution to the etiology of actinomycosis. The experimental production of actinomycosis in guinea pigs inoculated with the contents of carious teeth. Boston Med. Surg. J., **163**, 82–85.

(85) LORD, F. T. 1910 The etiology of actinomycosis. The presence of actinomycetes in the contents of carious teeth and the tonsillar crypts of patients without actinomycosis. J. Am. Med. Assoc., **55**, 1261–1263.

(86) LORD, F. T., AND TREVETT, L. D. 1936 The pathogenesis of actinomycosis. Recovery of actinomyces-like organisms from the normal mouth. J. Infectious Diseases, **58**, 115–120.

(87) LYONS, C., OWEN, C. R., AND AYERS, W. B. 1943 Sulfonamide therapy in actinomycotic infections. Surgery, **14**, 99–104.

(88) McKENTY, F. E. 1913 A study of cases of actinomycosis. Am. J. Med. Sci., **145**, 835–857.

(88A) MACKENZIE, I. 1931 Pathogenic Leptothriceae in, A System of Bacteriology in Relation to Medicine. Medical Research Council, London, **8**, 91–100.

(89) McWilliams, C. A. 1917 Actinomycosis of phalanx of finger. Annals Surg., **66**, 117–118.

(90) Magnusson, H. 1928 The commonest forms of actinomycosis in domestic animals and their etiology. Acta Path. Microbiol. Scand., **5**, 170–245.

(91) Magrou, J. 1919 Les formes actinomycotiques du staphylodoque. Ann. inst. Pasteur, **33**, 344–374.

(92) Mathieson, D. R., Harrison, R., Hammond, C., and Henrici, A. T. 1935 Allergic reactions of actinomycetes. Am. J. Hyg., **21**, 405–421.

(93) Mathis, H. 1938 Der supragingivale Zahnstein. Deut. Zahn-Mund-Kieferheilk., **5**, 114–127.

(94) Mendel, J. 1918 *Leptothrix asteroide*. Compt. rend. soc. biol., **81**, 471–475.

(95) Miller, W. D. 1890 Micro-Organisms of the Human Mouth. S. S. White Dental Mfg., Co., Philadelphia.

(96) Moore, B. 1910 The physiological and pathological metabolism of calcium and phosphates in relationship to the deposition of insoluble salts in formation of bones, teeth and calculi. Brit. Dental J., **31**, 825–831.

(97) Naeslund, C. 1925 Studies of actinomyces from the oral cavity. Acta Path. Microbiol. Scand., **2**, 110–140.

(98) Naeslund, C. 1925 Studien über Speichelsteinbildung. Acta Path. Microbiol. Scand., **2**, 244–276.

(99) Naeslund, C. 1926 Studies of tartar formation. Acta Path. Microbiol. Scand., **3**, 637–677.

(100) Naeslund, C. 1931 Experimentelle studien über die ätiologie und pathogenese der Aktinomykose. Acta Path. Microbiol. Scand., **8**, Suppl. 6, 1–156.

(101) Naeslund, C., und Dernby, K. G. 1923 Untersuchungen über einige physiologische Eigenschaften der Strahlenpilze. Biochem., **138**, 497–504.

(102) Negroni, P., and Bonfiglioli, H. 1937 Morphology and biology of *Actinomyces israeli* (Kruse, 1896). J. Trop. Med. Hyg., **40**, 226–232; 240–246.

(103) Neuber, E. 1940 Spezifische Diagnostik und Therapie der Aktinomyköse. Klin. Wochschr., **19**, 736–741.

(104) Northrop, P. M., and Crowley, M. C. 1943 The prophylactic use of sulfathiazole in transient bacteremia following the extraction of teeth. J. Oral Surg., **1**, 19–29.

(105) Parker, F., and Hudson, N. P. 1926 The etiology of Haverhill fever (Erythema arthriticum epidemicum). Am. J. Path., **2**, 357–379.

(106) Philipp, H. 1935 Über die Struktur von Zahnstein (Calculus dentalis supragingivalis). Z. Physiol. Chem., **223**, 209–214.

(107) Pinoy, E. 1913 Actinomyces et mycétomes. Bull. inst. Pasteur, **11**, 929–938; 977–984.

(108) Prinz, H. 1921 The origin of salivary calculus. Dental Cosmos, **63**, 231–238; 369–374; 503–510; 619–623.

(109) Puntoni, V. 1937 The relation of *Bacillus bifidus* (Lactobacillus) and the actinomycetic anaerobes, type Wolff-Israel. Ann. igiene, **47**, 157–168.

(110) Puntoni, V. 1939 La classificazione degli attinomiceti (microsyphonales vuill.). Third Intern. Congr. Microbiology, 195–196.

(111) Robin, C. 1847 Des Végétaux qui croissent sur l'Homme et sur les Animaux Vivants J. B. Baillière, Paris.

(112) Robin, C. 1853 Histoire Naturelle des Végétaux Parasites qui croissent sur l'Homme et sur les Animaux Vivants. J. B. Baillière, Paris.

(112A) Robinson, R. A. 1944 Actinomycosis of the subcutaneous tissue of the forearm secondary to a human bite. J. Am. Med. Assoc., **124**, 1049–1051.

(113) Rosebury, T. 1944 The aerobic non-hemolytic streptococci. Medicine, in press.

(114) Rosebury, T. 1944 The parasitic lactobacilli. In press.

(115) Rosebury, T., Epps, L. J., and Clark, A. R. 1944 A study of the isolation, cultivation and pathogenicity of *Actinomyces israeli* recovered from the human mouth and from actinomycosis in man. J. Infectious Diseases, **74**, 131–149.

(116) ROSEBURY, T., AND FOLEY, G. 1939 Experimental Vincent's infection. J. Am. Dental Assoc., **26,** 1798–1811.

(117) ROSEBURY, T., AND KARSHAN, M. 1938 Dental Science and Dental Art. S. M. Gordon. Lea & Febiger, Philadelphia, 250–268.

(118) ROSEBURY, T., AND WAUGH, L. M. 1939 Dental caries among Eskimos of the Kuskokwim area of Alaska. I. Clinical and bacteriologic findings. Am. J. Diseases Children, **57,** 871–893.

(119) SABIN, A. B. 1941 The filtrable microörganisms of the pleuropneumonia group. Bact. Rev., **5,** 331–335.

(120) ST. JOHN-BROOKS, R. 1931 The aerobic actinomyces. A System of Bacteriology in Relation to Medicine. Medical Research Council, London, **8,** 72–78.

(121) SALTZMANN, R. A. 1940 Relación del calcio y la actividad fosfatásica de la saliva con la formación del sarro y la velocidad de secretión. Rev. soc. argentina biol., **16,** 446–451.

(122) SAMPSON, W. E. A. 1932 Tristan da Cunba (Surgeon Lt. Commander Sampson's Report). Dental Surg., **29,** 219.

(123) SCHOTTMÜLLER, H. 1914 Zur Aetiologie und Klinik der Bisskrankheit (Ratten-Katzen-Eichhörnchen-Bisskrankheit). Dermatol. Wochschr., **58,** Suppl. 77–103.

(124) SHAHAN, M. S., AND DAVIS, C. L. 1942 The diagnosis of actinomycosis and actinobacillosis. Am. J. Vet. Research, **3,** 321–328.

(125) SIEGEL, E. 1935 Aktinomyzeten als Zahnsteinbildner. Arch. Hyg. Bakt., **113,** 223–233.

(126) SLACK, J. 1942 The source of infection in actinomycosis. J. Bact., **43,** 193–209.

(127) SMITH, G. H. 1930 Factors affecting the deposition of dental calculus. Australian J. Exptl. Biol. Med. Sci., **7,** 45–77.

(128) SÖDERLUND, G. 1927 Die Speichelsteinkrankheit ("Sialolithiasis") und ihr Verhalten zu der primären und duktogenen Speicheldrüsenaktinomykose. Acta Chir. Scand., **63,** Suppl. 9.

(129) STEINBACH, M. 1935 Agents of Disease and Host Resistance, *Gay et al.* C. C. Thomas, Springfield, p. 977.

(130) SULLIVAN, H. R., AND GOLDSWORTHY, N. E. 1940 A comparative study of anaerobic strains of *actinomyces* from clinically normal mouths and from actinomycotic lesions. J. Path. Bact., **51,** 253–261.

(131) TALBOT, E. S. 1913 Interstitial Gingivitis and Pyorrhea alveolaris. The Ransom & Randolph Co., Toledo.

(132) TASCHER, P. J., AND WAGREICH, P. 1943 Salivary phosphatase, relationship of, to calculus deposition. Senior thesis, School of Dental and Oral Surgery, Columbia University, N. Y.

(133) TAYLOR, W. E. 1934 Association of bacteria with dental calculus. J. Dental Research, **14,** 237.

(134) TENENBAUM, B., AND KARSHAN, M. 1937 Factors in human saliva correlated with the occurrence of salivary calculus. J. Am. Dental Assoc., **24,** 1255–1259.

(135) TENENBAUM, B., AND KARSHAN, M. 1939 Factors in saliva correlated with the occurence of calculus. J. Am. Dental Assoc., **26,** 1965–1971.

(136) TENENBAUM, B., AND KARSHAN, M. 1944 The composition and formation of salivary calculus. J. Periodontol., In press.

(137) THJØTTA, TH., HARTMANN, O., AND BOE, J. 1939 A study of *Leptotrichia trevisan.* History, morphology, biological and serological characteristics. Oslo, 1 Kommisjon Hos Jacob Dybwad, 1–41.

(138) TOPLEY, W. W. C., AND WILSON, G. S. 1937 The Principles of Bacteriology and Immunity. Wm. Wood & Co., New York, 2nd ed.

(139) TREVISAN, V. 1879 Cited by Buchanan, R. E., General Systematic Bacteriology. Williams & Wilkins Co., Baltimore, 1925, p. 352.

(140) TUNNICLIFF, R. 1926 The organism of actinomyces-like tonsillar granules. J. Infectious Diseases, **38**, 366–370.

(141) TUNNICLIFF, R., AND JACKSON, L. 1930 *Vibriothrix tonsillaris*. The organism of actinomyces-like tonsillar granules. J. Infectious Diseases, **46**, 12–17.

(142) VICENTINI, F. 1897 Bacteria of the Sputa and Cryptogamic Flora of the Mouth. Baillière, Tindall and Cox, London. Cited by Beust, T. B., 1937 J. Dental Research, **16**, 379–386.

(143) WAKSMAN, S. A. 1940 On the classification of actinomycetes. J. Bact., **39**, 549–558.

(144) WAKSMAN, S. A. 1941 Antagonistic relations of microörganisms. Bact. Rev., **5**, 231–291.

(145) WAKSMAN, S. A., AND HENRICI, A. T. 1943 The nomenclature and classification of the actinomycetes. J. Bact., **46**, 337–341.

(146) WALKER, O. 1938 Sulphanilamide in the treatment of actinomycosis. Lancet **234**, 1219–1220.

(147) WARREN, J. 1942 Observations on some biological characteristics of organisms of the pleuropneumonia group. J. Bact., **43**, 211–228.

(148) WARWICK, W. T. 1923 A clinical contribution to the aetiology of actinomycosis. Lancet **205**, 497–501.

(149) WHERRY, W. B., AND OLIVER, W. W. 1916 *Leptothrix innominata* (Miller). J. Infectious Diseases, **19**, 299–303.

(150) WILKINSON, E. E. 1941 Actinomycosis treated with sulfanilamide. J. Pediatrics, **18**, 805–810.

(151) WILLSMORE, N. M. 1937 A study of the osmotic pressure and viscosity of saliva and their relation to common oral conditions. Australian J. Dentistry, **41**, 161–195.

(152) WOLFF, M., UND ISRAEL, J. 1891 Ueber Reincultur des Actinomyces und seine Uebertragbarkeit auf Thiere. Virchow's Arch., **126**, 11–59.

(153) WRIGHT, J. H. 1905 The biology of the microörganism of actinomycosis. J. Med. Research, **13**, 349–404.

(154) WRIGHT, J. H. 1925 Modern Medicine (Osler, W., McCrae, T., Funk, E. H.) Lea & Febiger, Philadelphia. Chapter 6, 783–798.

(155) ZANDER, H. A. 1941 The distribution of phosphatase in gingival tissue. J. Dental Research, **20**, 347–353.